Use of milk fatty acids composition to discriminate area of origin of bulk milk

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ABSTRACT

The fatty acid composition of cow milk, collected in a survey from 19 dairy farms in the border area between Italy and Slovenia, was investigated for 2 consecutive years (2005 and 2006) to assess the possibility of discriminating the area of the origin of the milk. Farms were selected based on diet, animal breed, and farm management to represent the local variability of the systems. In Slovenian farms, grass silage and hay prevailed over corn silage and concentrate feeds, whereas in Italian farms, hay and concentrates were the predominant components of the diet. Fifty-three fatty acids were separated and quantified in Italian and Slovenian milks. Saturated fatty acids represented the most abundant class, followed by monounsaturated and polyunsaturated fatty acids. Significant differences were observed between Italian and Slovenian milks for the concentration of 40 fatty acids, whereas significant differences were observed between years of production for 15 fatty acids. Discriminant analysis was used to identify a classification criterion of milk, using country and year of production as grouping variables. Considering statistical results and the scatter plot of the scores of the first 2 functions, the best discriminant criteria were those based on unsaturated fatty acids and on fatty acids with several carbon atoms ≥ 18 .

Key words: milk fatty acid, grass silage, n-3, n-6

INTRODUCTION

Lipid content and fatty acid profile are considered to have an important role in the human diet and there is increasing interest in improving the fatty acid (FA) profile of milk to obtain a product with positive functions for human health. The investigation of farming and dietary conditions affecting the fatty acid profile is becoming more important to increase the added value of local products.

Monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) are known to be valuable compounds in human nutrition against cardiovascular disease, cancer, and inflammatory and autoimmune diseases. Conjugated linoleic acid (CLA) and n-3 PUFA are considered the major compounds present in milk fat with activity against these diseases (Ulbricht and Southgate, 1991; Ip et al., 1997; Parodi, 1999).

Fatty acids in milk derive either from long-chain fatty acids contained in the diet or from fat mobilized from body stores and from microbial synthesis (Kennelly, 1996). Furthermore, approximately 50% of the short- and medium-chain compounds derive from de novo synthesis at the mammary gland level and depend on acetate and butyrate provision and genetics (Palmquist et al., 1993; Mele et al., 2009). Small amounts of CLA are synthesized by ruminants, whereas n-3 and n-6 PUFA have to be ingested with feed to be secreted in milk (Elgersma et al., 2006).

After hydrolyzation by microbial enzymes, lipids contained in the diet are biohydrogenated from unsaturated (C18:3, C18:2) to saturated fatty acids (C18:0) through the formation of the intermediate C18:2 *cis*-12, *trans*-11 (Jenkins, 1993; Kennelly, 1996), with the exception of linoleic acid, which is first isomerized to C18:2 *cis*-9, *trans*-12. The extent and completion of the biohydrogenation process depends on rumen condition and diet composition, is promoted by the presence of cell-free ruminal fluid and the size of feed particles, and is inhibited by large amount of linoleic acid (Jenkins, 1993). In the latter case, a variable amount of *trans* isomers escape biohydrogenation and can be found in milk fat (Wu et al., 1991). Therefore, the FA composition of milk fat can be modified by feeding lipid sources that alter the FA profile of lipids leaving the rumen (Kennelly, 1996; Chilliard et al., 2001; Elgersma et al., 2004), by changing the composition and localization of pasture (Collomb et al., 2002) or animal management (Jahreis et al., 1997), and by some internal factors such as animal genetics (Malacarne et al., 2001). All these factors can vary considerably in relation to geographical origin and can influence chemical composition of milk fat.

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Table 1. Estimated characteristics of herds and farms in Italy (Friuli Venezia Giulia region) and Slovenia during 2005 and 2006

Characteristic	Italy		Slovenia	
	2005	2006	2005	2006
Utilized agricultural area (UAA), ha	28,642	28,708	95,491	94,220
Dairy cattle (bulls, cows, heifers), ¹ n	54,649	52,823	130,990	124,682
Dairy cows, ¹ n	44,816	44,113	111,424	106,039
Stocking density (dairy cattle/UAA), n/ha	1.91	1.84	1.37	1.32
Stocking density (cows/UAA), n/ha	1.56	1.54	1.17	1.12
Yearly milk production, kg/yr	7,510	7,620	5,670	5,803
Animal breed, %				
Brown Swiss	5.52	5.38	17.70	16.90
Holstein-Friesian	45.20	46.21	28.80	30.40
Simmental	42.95	41.64	48.40	47.30
Others	6.32	6.77	5.00	5.30

¹Animals ≥ 2 yr old.

The present survey was carried out in the border area between Italy and Slovenia, 2 European countries territorially contiguous but different in terms of economics and culture. The aim of the study was to assess the ability to discriminate the area of the origin of the milk using differences in the FA profile of bulk milk. To accomplish this, milk samples were collected from dairy farms located in the border area between Slovenia and Italy for 2 consecutive years (2005 and 2006).

MATERIALS AND METHODS

Experimental Description

Nineteen dairy farms located in the border area between Italy and Slovenia were selected from the local breeder associations based on factors such as diet, animal breed, and level of milk production to represent local productive variability (Table 1). Nine farms were located in the Friuli Venezia Giulia region (Italy) within

the provinces of Udine, Gorizia, and Trieste, and 10 farms were located in the regions of Obalno-Kraska and Goriska in Slovenia. From February to April of 2 consecutive years, samples of feed rations and bulk milk were collected from each farm. Cows belonged predominantly to Holstein-Friesian, Simmental, and Brown Swiss breeds and all were housed in free-stalls. The main characteristics of herds and farms used in the study are reported in Table 2.

Rations

Depending on farm management, feeds were administered as TMR ad libitum or separated (corn and hay or lucerne). During the experimental period, animals within each stable were fed with the same rations and had no access to grazing. The composition of rations did not change significantly between 2005 and 2006, but was substantially different between countries (Table 3). In Slovenian farms, grass silage was the predominant

Table 2. Main characteristics of herds and farms used in the study

Characteristic	Italy		Slovenia		2005		2006	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Utilized agricultural area (UAA), ha	55.78	61.87	65.00	52.11	59.97	57.85	59.97	57.85
Animals (cows, bulls, steers), n	76.50	59.07	93.85	91.68	86.95	78.68	81.82	73.33
Cows in lactation, n	42.79	31.57	57.80	64.06	50.73	47.92	48.50	51.21
Stocking density (animals/UAA), n/ha	2.24	2.66	1.52	0.84	1.96	2.18	1.87	1.97
Stocking density (cows/UAA), n/ha	1.31	1.42	0.85	0.54	1.15	1.25	1.04	1.01
Days of lactation, n	187.36	22.42	169.66	31.89	176.46	34.55	179.63	22.67
Daily milk production, kg/d	23.50	3.87	17.94	2.60	21.44	4.33	20.31	4.35
Daily milk production/UAA, kg/d/ha	0.81	0.95	0.48	0.34	0.56	0.45	0.76	0.97
Yearly milk production, kg/yr	7,411.59	804.60	5,565.29	948.03	6,560.18	1,305.47	6,504.62	1,270.15
Yearly milk production/UAA, kg/yr/ha	320.76	420.50	146.26	102.09	240.90	317.38	241.97	347.77
Animal breed								
Brown Swiss	8.67	27.92	13.60	20.77	11.32	25.93	10.50	24.16
Holstein-Friesian	43.88	62.81	44.45	95.80	46.41	81.02	41.86	77.77
Simmental	39.17	42.48	30.40	54.42	33.41	47.36	36.95	49.46
Crossbreeds	5.54	10.59	5.35	7.44	5.77	9.21	5.14	9.37

Table 3. Dry matter intake and composition of rations collected in Italian and Slovenian dairy farms during 2005 and 2006

Item	Italy		Slovenia		2005		2006		P-value ¹		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	C	Y	C × Y
DMI, kg/d	21.12	1.19	18.09	3.49	19.59	2.65	19.60	2.88	***	NS	NS
Ration											
Hay, lucerne, g/kg of DM	426.90	169.20	164.70	172.85	313.80	201.80	307.60	236.60	***	NS	NS
Corn silage, g/kg of DM	168.20	151.90	52.30	91.20	118.00	137.60	122.10	145.00	**	NS	NS
Grass silage, g/kg of DM	—	—	567.30	176.20	230.50	296.00	237.30	303.20	***	NS	NS
Concentrate feeds, g/kg of DM	404.90	79.60	215.70	95.20	337.70	113.10	332.90	131.30	***	NS	NS
Forages (F), %	59.50	8.00	78.40	10.50	66.20	11.30	66.70	13.10	***	NS	NS
Concentrates (C), %	40.50	8.00	21.60	10.50	33.80	11.30	33.30	13.10	***	NS	NS
F:C	1.58	0.57	3.97	2.35	2.43	1.57	2.77	2.34	***	NS	NS
Chemical analysis											
DM, g/kg	700.50	160.40	611.50	96.00	644.80	123.90	642.20	149.10	**	NS	NS
CP, g/kg of DM	136.40	18.40	127.80	26.30	131.20	21.20	131.00	21.60	*	NS	NS
Ether extract, g/kg of DM	28.90	9.20	29.10	10.30	29.20	9.70	29.20	9.50	NS	NS	NS
NDF, g/kg of DM	403.40	46.70	454.90	85.20	422.90	68.30	423.60	70.40	*	NS	NS
Starch, g/kg of DM	218.70	38.00	145.80	115.20	184.20	66.20	194.40	80.00	**	NS	NS
Ash, g/kg of DM	69.00	6.30	69.10	21.50	66.80	10.10	66.70	9.90	NS	NS	NS

¹Effects of C (country of origin), Y (year of production), and C × Y (their interaction).

²Mean values of grass silage in 2005 and 2006 are calculated as the average between Italian and Slovenian values.

³Cereals and integrators of proteins, vitamins, and minerals.

⁴Hay, lucerne, corn, and grass silage.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

component of the ration compared with the percentages of grass hay, corn silage, and concentrate feeds. On Italian farms, grass hay and concentrate feeds represented the predominant components, and grass silage was not used.

A week before bulk milk collection, individual feeds and TMR were sampled on each farm and analyzed for DM, CP, lipids (ether extract), ash, NDF (Goering and Van Soest, 1970), and starch (Martillotti et al., 1987). For 4 consecutive weeks before the collection of milk samples, the average amount of feed consumed was recorded once a week as the difference between the mean amount offered to the cows and the mean amount refused daily. The average daily DMI was estimated as the mean value of the 4 observations. The mean formulation and chemical composition of rations are reported in Table 3.

Milk Collection

On each farm, 3 samples of bulk milk were collected from the cooled tank in 50-mL sterile vials (Sarstedt s.r.l., Verona, Italy) and transferred within 2 h to the laboratory. Then, 2 samples were immediately analyzed for fat, protein, lactose, and DM content by means of infrared spectrophotometry (Table 4), and the remaining sample was stored at -70°C until FA composition analysis.

FA Composition

Thirty-eight milk samples (18 Italian and 20 Slovenian) were analyzed for FA composition. For analysis, stored milk samples were thawed at 37°C , thoroughly mixed, and used for preparation of fatty acid methyl es-

Table 4. Chemical composition of milk produced in Italian and Slovenian farms during 2005 and 2006

Item	Italy		Slovenia		2005		2006		P-value ¹		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	C	Y	C × Y
Observations, n	18		20		19		19				
Fat, %	3.82	0.35	3.59	0.55	3.56	0.55	3.84	0.33	NS	NS	NS
Protein, %	3.43	0.18	3.30	0.18	3.37	0.14	3.36	0.23	*	NS	NS
Lactose, %	4.89	0.08	4.92	0.14	4.92	0.13	4.90	0.11	NS	NS	NS
DM, %	9.07	0.22	8.98	0.25	9.00	0.21	9.03	0.26	NS	NS	NS

¹Effects of C (country of origin), Y (year of production), and C × Y (their interaction).

* $P < 0.05$.

Table 5. Mean concentration of fatty acids detected in milk of Italian and Slovenian farms during 2005 and 2006

FA, g/100 g of total FA	Italy		Slovenia		2005		2006		<i>P</i> -value ¹		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	C	Y	C × Y
Observations, n	18		20		19		19				
Saturated fatty acids											
C16:0	32.42	1.55	31.45	2.62	31.04	2.50	32.78	1.48	NS	*	NS
C18:0	10.59	1.79	10.23	1.06	10.69	1.52	10.11	1.33	NS	NS	NS
C14:0	12.17	1.07	12.18	0.78	12.23	1.13	12.12	0.66	NS	NS	NS
C12:0	3.79	0.48	3.65	0.35	3.82	0.42	3.62	0.40	NS	NS	NS
C10:0	2.99	0.32	2.96	0.30	3.07	0.32	2.88	0.26	NS	NS	NS
C6:0	1.52	0.14	1.60	0.16	1.67	0.13	1.45	0.08	*	***	NS
C15:0	1.17	0.13	1.28	0.19	1.21	0.15	1.25	0.20	*	NS	NS
C8:0	0.97	0.17	1.20	0.14	1.08	0.26	1.10	0.08	***	NS	***
C17:0	0.58	0.04	0.71	0.13	0.64	0.11	0.66	0.13	***	NS	NS
C15:0 <i>anteiso</i>	0.48	0.04	0.60	0.08	0.56	0.08	0.54	0.09	***	NS	NS
C17:0 <i>anteiso</i>	0.45	0.04	0.54	0.07	0.50	0.07	0.50	0.08	***	NS	NS
C17:0 <i>iso</i>	0.44	0.09	0.39	0.23	0.26	0.10	0.57	0.06	***	***	***
C16:0 <i>iso</i>	0.32	0.05	0.36	0.05	0.34	0.05	0.34	0.05	*	NS	*
C15:0 <i>iso</i>	0.30	0.02	0.38	0.06	0.35	0.07	0.33	0.06	***	NS	NS
C20:0	0.15	0.02	0.18	0.03	0.16	0.03	0.17	0.03	***	NS	NS
C13:0	0.14	0.02	0.13	0.03	0.13	0.02	0.14	0.03	NS	NS	NS
C11:0	0.07	0.02	0.04	0.02	0.05	0.02	0.05	0.03	***	NS	NS
C18:0 <i>iso</i>	0.06	0.01	0.08	0.02	0.07	0.02	0.07	0.02	***	*	NS
C22:0	0.06	0.01	0.08	0.02	0.07	0.02	0.07	0.02	***	NS	NS
C24:0	0.04	0.01	0.06	0.01	0.05	0.01	0.05	0.02	***	NS	NS
C23:0	0.03	0.01	0.04	0.02	0.03	0.01	0.03	0.02	**	NS	NS
C21:0	0.03	0.00	0.04	0.01	0.03	0.01	0.03	0.01	*	NS	NS
C16:0 <i>anteiso</i>	0.01	0.00	0.02	0.01	0.02	0.01	0.02	0.00	***	***	***
C18:0 <i>anteiso</i>	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	NS	***	**
Total	68.79	1.99	68.21	2.53	68.08	2.81	68.89	1.56	NS	NS	NS
Monounsaturated fatty acids											
ΣC18:1 ²	23.65	1.85	23.67	2.27	24.03	2.51	23.29	1.44	NS	NS	NS
C16:1n-7	1.50	0.15	1.60	0.11	1.53	0.13	1.57	0.14	*	NS	NS
C14:1n-5	0.91	0.13	1.03	0.10	0.96	0.14	0.98	0.11	**	NS	NS
C17:1n-7	0.32	0.07	0.23	0.03	0.27	0.07	0.28	0.08	***	NS	NS
C10:1	0.24	0.03	0.27	0.02	0.26	0.04	0.25	0.03	***	NS	NS
C20:1n-12 + n-15	0.15	0.02	0.18	0.03	0.16	0.03	0.17	0.02	**	NS	NS
C13:1n-1	0.14	0.03	0.18	0.03	0.17	0.03	0.16	0.04	***	NS	NS
C12:1n-1	0.13	0.02	0.16	0.02	0.14	0.02	0.14	0.03	***	NS	**
C20:1n-9	0.04	0.01	0.05	0.01	0.04	0.01	0.05	0.01	NS	***	NS
C15:1n-5	0.02	0.00	0.01	0.00	0.01	0.00	0.02	0.00	*	**	NS
C21:1n-9	0.01	0.02	0.01	0.01	0.00	0.00	0.02	0.01	***	***	***
C22:1n-9	0.01	0.02	0.01	0.01	0.00	0.00	0.02	0.01	***	***	***
C24:1n-9	0.01	0.00	0.02	0.00	0.02	0.00	0.01	0.00	***	**	NS
Total	27.13	1.69	27.42	2.25	27.59	2.46	26.96	1.34	NS	NS	NS
n-6 polyunsaturated fatty acids											
C18:2n-6 <i>cis</i> -9,12	2.67	0.35	2.11	0.27	2.41	0.39	2.33	0.45	***	NS	NS
C20:4n-6	0.19	0.01	0.15	0.03	0.16	0.03	0.17	0.03	***	NS	NS
C20:3n-6	0.14	0.02	0.08	0.02	0.11	0.04	0.11	0.03	***	NS	NS
C18:3n-6	0.06	0.02	0.04	0.01	0.06	0.02	0.04	0.01	***	***	**
C22:4n-6	0.05	0.02	0.03	0.01	0.03	0.02	0.04	0.02	**	NS	NS
C22:2n-6	0.03	0.01	0.04	0.02	0.03	0.02	0.04	0.02	NS	NS	NS
C20:2n-6	0.03	0.00	0.03	0.01	0.03	0.01	0.03	0.01	NS	NS	NS
Total	3.17	0.37	2.48	0.28	2.83	0.45	2.76	0.50	***	NS	NS
n-3 polyunsaturated fatty acids											
C18:3n-3	0.44	0.10	0.81	0.21	0.64	0.25	0.62	0.24	***	NS	NS
C22:5n-3	0.07	0.01	0.12	0.03	0.10	0.03	0.10	0.03	***	NS	NS
C20:5n-3	0.04	0.01	0.08	0.02	0.06	0.02	0.06	0.03	***	NS	NS
C22:3n-3	0.00	0.01	0.00	0.01	0.01	0.01	0.00	0.00	NS	**	NS
C18:4n-3	0.01	0.00	0.02	0.00	0.01	0.00	0.02	0.00	***	NS	NS
C20:3n-3	0.01	0.01	0.00	0.01	0.01	0.01	0.00	0.00	***	***	***
C22:6n-3	0.01	0.00	0.02	0.00	0.02	0.00	0.01	0.01	**	NS	NS
Total	0.58	0.12	1.05	0.25	0.85	0.31	0.81	0.29	***	NS	NS

Continued

Table 5 (Continued). Mean concentration of fatty acids detected in milk of Italian and Slovenian farms during 2005 and 2006

FA, g/100 g of total FA	Italy		Slovenia		2005		2006		<i>P</i> -value ¹		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	C	Y	C × Y
Stereoisomers of conjugated linoleic acid											
C18:2 <i>cis</i> -9, <i>trans</i> -11	0.46	0.10	0.69	0.24	0.63	0.28	0.54	0.13	***	NS	NS
C18:2 <i>trans</i> -10, <i>cis</i> -12	0.01	0.01	0.01	0.01	0.00	0.00	0.01	0.00	NS	***	NS
Total	0.47	0.10	0.70	0.24	0.63	0.28	0.55	0.13	***	NS	NS
n-6/n-3 ratio	6.28	1.20	3.03	1.01	4.07	2.07	4.09	1.81	***	NS	NS

¹Effects of C (country of origin), Y (year of production), and C × Y (their interaction).

²Sum of C18:1 *cis* and *trans* isomers.

P* < 0.05; *P* < 0.01; ****P* < 0.001.

ters, without prior extraction of fat from sample (Park and Goins, 1994).

Fatty acid methyl esters were separated by using an Agilent 6890 series GC instrument equipped with an Agilent 7683 Automatic Liquid Sampler (Agilent Technologies, Palo Alto, CA), a split injector, a flame-ionization detector, and an Omegawax 320 fused-silica capillary column (30 m × 0.25 μm × 0.32 mm i.d., Supelco, Bellefonte, PA) was used. Agilent GC ChemStation was used for data acquisition and processing. Individual fatty acid methyl esters were identified and quantified using external methyl ester standard mixtures (Nu-Chek Prep Inc., Elysian, MN). Analytical results were expressed in grams of FA/100 grams of FA.

Statistical Analysis

Fatty acids were considered individually and grouped in classes as follows: saturated fatty acids (**SFA**), unsaturated fatty acids (**UFA**), MUFA, n-3 PUFA, n-6 PUFA, and CLA. The differences of FA concentration in milk samples from Italian and Slovenian farms were evaluated using the multivariate general linear mixed model with country and year as fixed factors (SPSS, 1997).

The classification of milk samples in 4 groups (depending on country of origin and year of production) was evaluated with discriminant analysis (SPSS, 1997), using different classes of FA as a set of independent variables: all FA (*n* = 53), SFA (*n* = 24), UFA (*n* = 29), and long-chain FA (**LCFA**) with C atoms ≥18 (*n* = 30).

RESULTS AND DISCUSSION

Milk Chemical Composition

The overall means, SD, and *P*-values of milk produced in Italian and Slovenian farms during 2005 and

2006 are reported in Table 4. Only protein content differed significantly between countries, with lower protein content in milk from Slovenian farms, probably related to the lower fermentable carbohydrates and higher fiber contents in the rations of the Slovenian dairy cows (Table 3).

Milk FA Composition

The percentage of all measured FA in Italian and Slovenian milk collected in 2005 and 2006 are reported in Table 5. Fifty-three FA (24 SFA, 12 MUFA + sum of 18:1 isomers, 14 n-3 and n-6 PUFA, and 2 CLA) were separated and quantified for Italian and Slovenian milk samples and over both years.

As expected, SFA represented the most abundant class, followed by MUFA and PUFA. The most abundant FA in cow milk were C16:0, sum of C18:1 isomers, C18:0, and C14:0, in agreement with results of Malacarne et al. (2001). No statistical differences in the concentrations of these compounds were observed between Italian and Slovenian milks. Also, short- and medium-chain SFA, such as C6:0, C8:0, C10:0, and C12:0, were present at the expected levels (Malacarne et al., 2001; Jones et al., 2005).

The remaining SFA (C20:0, C22:0, and C24:0) and odd- and branched-chain saturated fatty acids (**OBCFA**; C11:0, C13:0, C15:0, C15:0 *iso*, C15:0 *anteiso*, C16:0 *iso*, C16:0 *anteiso*, C17:0, C17:0 *iso*, C17:0 *anteiso*, C18:0 *iso*, C18:0 *anteiso*, C21:0, and C23:0) represented 4.09% and 4.62% of total SFA in Italy and Slovenia, respectively, with most being higher in Slovenian milk. The main OBCFA in milk of dairy cows are isomers of tetradecanoic acid (C14:0 *iso*), pentadecanoic acid (C15:0, C15:0 *iso*, and C15:0 *anteiso*), hexadecanoic acid (C16:0 *iso*), and heptadecanoic acid (C17:0, C17:0 *iso*, and C17:0 *anteiso*); their presence in milk fat largely originates from bacteria leaving the rumen (Keeney et al., 1962). Vlaeminck et al. (2006) reported that increasing forage:concentrate ratio (**F:C**)

in the diet resulted generally in an higher proportion of milk OBCFA because of an increase in cellulolytic bacteria. Also, changes in forage type (corn silage vs. grass silage) that cause an increase in dietary starch and a decrease of NDF (Nielsen et al., 2004; Shingfield et al., 2005) can lead to variations in rumen microbial population and consequently to a decrease in milk OBCFA (Vlaeminck et al., 2006). These observations are consistent with our results. Higher levels of OBCFA in Slovenian milk compared with Italian milk could be the consequence of the higher F:C (Table 3) caused by the use of grass silage and the very low proportion of corn silage.

Among MUFA, C18:1 isomers were the main components. Altogether, the remaining monounsaturated compounds formed 3.48% (Italy) and 3.75% (Slovenia) of total milk fat and all were significantly higher in Slovenian milk, with the exception of C20:1n-9 and C15:1n-5.

The PUFA represented 4.22 and 4.23% of total fat in Italian and Slovenian milks, respectively, and C18:2n-6 was the predominant compound. Except for C20:2n-6, C22:2n-6, C22:3n-3, and C18:2 *trans*-10, *cis*-12, the percentages of the remaining PUFA differed significantly between milk produced in Italy and Slovenia. The percentages of n-3 PUFA were higher in Slovenian milk samples (1.05 vs. 0.58%), whereas the percentages of n-6 PUFA (3.17 vs. 2.48%) and the ratio between n-6 and n-3 PUFA (6.28 vs. 3.03) were higher in Italian than in Slovenian milk samples.

Within PUFA, 2 isomers of CLA were measured. Significantly higher concentrations of C18:2 *cis*-9, *trans*-11 were observed in Slovenian milk samples compared with Italian milk samples, whereas concentrations of C18:2 *trans*-10, *cis*-12 were lower and similar in both Slovenian and Italian milk samples.

The results of the analysis indicated clear variations of FA composition of bulk milk collected in 2 contiguous countries. Because Slovenian milk contained an higher percentage of n-3 PUFA and CLA, a family of compounds known to have positive effects on human health (Tricon et al., 2004; Benjamin and Spener, 2009; Calder and Yaqoob, 2009), it is likely that this milk is more suitable for healthy human nutrition compared with the milk produced in Italy.

Considering previous studies (Grummer, 1991; Chilliard et al., 2001; Whiting et al., 2004) relating the differences of milk fatty acid composition to diet characteristics, the observed differences of milk fatty acid concentrations between the 2 countries could be caused by the different rations used for feeding. As reported above, basal diets fed to lactating cows differed substantially between Italy and Slovenia. The percentage of forage in the diets was 59.50 and 78.40%

in Italy and Slovenia, respectively (Table 3). Despite the amount of UFA contained in plant tissue (Hawke, 1973), 75 to 90% of linoleic acid and 85 to 95% of linolenic acid generally undergoes biohydrogenation in the rumen (Kalscheur et al., 1997; Lock and Garnsworthy, 2002; Loor et al., 2004). However, because of the high F:C of Slovenian rations, a significant amount of UFA probably bypassed rumen biohydrogenation and was absorbed in the intestine. Slovenian rations also contained higher percentages of grass silage and lower percentages of corn silage. Grass and grass silage are a primary source of n-3 PUFA for ruminants, especially linolenic acid (Fievez et al., 2004). During harvesting of hay, the contents of FA, especially linolenic acid, are greatly reduced. On the contrary, in fresh grasses and in properly prepared grass silages, the contents of FA and linolenic acid remain almost unchanged (Doreau and Poncet, 2000; Chilliard et al., 2001). On Italian farms, the main components of diet were hay (42.69%), corn silage (16.82%), and concentrate (40.49%) based on corn grain, whereas grass silage was not used. Because forages such as grass and grass silages contain primarily linolenic acid, whereas corn grain contains mainly linoleic acid (Elgersma et al., 2006), the significant differences in n-3 and n-6 PUFA and CLA contents observed in Italian and Slovenian milk could be related to the remarkable differences in the diets.

Year of milk collection significantly affected the concentrations of 6 SFA (C6:0, C16:0, C16:0 *anteiso*, C17:0 *iso*, C18:0 *iso*, and C18:0 *anteiso*), 5 MUFA (C15:1n-5, C20:1n-9, C21:1n-9, C22:1n-9, and C24:1n-9), and 4 PUFA (C18:3n-6, C18:2 *trans*-10, *cis*-12, C20:3n-3, and C22:3n-3).

Ten fatty acids were significantly different for the interaction between country and year, indicating that these compounds can vary from year to year in the 2 areas. During 2005, C8:0, C12:1n-1, C16:0 *anteiso*, and C21:1n-9 were higher in Slovenian than in Italian milk (1.29 vs. 0.83%, 0.15 vs. 0.14%, 0.03 vs. 0.01%, and 0.04 vs. 0.03%, respectively); C17:0 *iso*, C18:0 *anteiso*, C18:3n-6, C20:3n-3, and C22:1n-9 were higher in Italian than in Slovenian milk (0.36 vs. 0.17%, 0.02 vs. 0.01%, 0.08 vs. 0.05%, 0.02 vs. 0.00%, and 0.001 vs. 0.000%, respectively); and C16:0 *iso* presented the same mean concentration in both countries (0.34%). In 2006, C12:1n-1, C16:0 *iso*, C17:0 *iso*, C18:0 *anteiso*, and C21:1n-9 had a higher concentration in Slovenian than in Italian milk (0.16 vs. 0.11%, 0.37 vs. 0.31%, 0.61 vs. 0.52%, 0.01 vs. 0.00%, and 0.06 vs. 0.05%, respectively); C18:3n-6 was higher in the Italian milk (0.05 vs. 0.04%); and C8:0, C16:0 *anteiso*, C20:3n-3, and C22:1n-9 presented the same mean concentrations in both Italian and Slovenian milks (1.10, 0.02, 0.00, and 0.003%, respectively).

Figure 1. Classification of milk samples according to country of origin and year of production with discriminant analysis. Comparison of scatter plots obtained using all fatty acids (1), saturated fatty acids (2), unsaturated fatty acids (3), or long-chain fatty acids with several carbon atoms ≥ 18 (4). $\times$ = milk sample; $\blacksquare$ = Italian milk collected in 2005; $\square$ = Italian milk collected in 2006; $\bullet$ = Slovenian milk collected in 2005; $\circ$ = Slovenian milk collected in 2006.

Because the composition of forages could modify the FA composition of milk (Elgersma et al., 2006) and the lipid content of plants could be considerably influenced by factors such as plant species, growth stage, temperature, and light intensity (Hawke, 1973), the differences observed for fatty acids composition of milk in different years could be the consequence of annual variations of micro-environmental conditions and botanical composition of pasture plants. Despite the similar climatic characteristics (Dalla Marta et al., 2006), Slovenia and Friuli Venezia Giulia present weather conditions that are extremely variable (Poldini and Feoli, 2001; Česen et al., 2006).

Discriminant Analysis

To evaluate if the concentrations of FA in milk fat reflected the origin of milk, a posterior classification of milk samples into 4 groups (2 countries of origin $\times$ 2 years of sampling) was performed using discriminant analysis. The general relationship between the 4 groups is illustrated in Figure 1. The axes correspond to the first 2 canonical discriminant functions, that are the 2 linear combinations of variables that best separate the defined groups. The coefficients for each variable in the function are chosen to maximize the ratio of the between-groups sums of squares to the within-groups

Table 6. Statistical results of discriminant analysis of milk samples collected in Italian (IT) and Slovenian (SL) dairy farms in 2005 and 2006 using different fatty acids classes as classification criterion

Variables used in the model ¹	Variables effectively included in the model ²	Original									Discriminant functions						
		Milk samples in the correct group, n				Milk samples misplaced, n					Correctly classified, %	Explained variance, %			Wilk's lambda for test functions ³		
		2005		2006		2005		2006									
		IT	SL	IT	SL	IT	SL	IT	SL	IT		SL	1	2	3	1-3	2-3
FA (n = 53)	C6:0; C8:0; C13:1n-1; C14:0; C14:1n-5; C15:0 <i>iso</i> ; C15:0 <i>anteiso</i> ; C15:0; C15:1n-5; C16:0 <i>iso</i> ; C16:0 <i>anteiso</i> ; C16:0; C16:1n-7; C17:0 <i>iso</i> ; C17:0 <i>anteiso</i> ; C17:0; C17:1n-7; C18:0 <i>iso</i> ; C18:0 <i>anteiso</i> ; C18:0; Σ C18:1; C18:2 <i>c9 t11</i> ; C18:2 <i>t10 c11</i> ; C18:2n-6; C18:3n-6; C18:3n-3; C18:4n-3; C20:0; C20:1n-12+15; C20:2n-6; C20:3n-6; C22:5n-3; C24:0.	9	10	9	10	—	—	—	—	100.0	79.0	16.3	4.7	0.000	0.001	0.044	
SFA (n = 24)	C6:0; C8:0; C10:0; C11:0; C12:0; C13:0; C14:0; C15:0 <i>iso</i> ; C15:0 <i>anteiso</i> ; C15:0; C16:0 <i>iso</i> ; C16:0 <i>anteiso</i> ; C16:0; C17:0 <i>iso</i> ; C17:0 <i>anteiso</i> ; C17:0; C18:0 <i>iso</i> ; C18:0 <i>anteiso</i> ; C18:0; C20:0; C21:0; C22:0; C23:0; C24:0.	9	10	9	10	—	—	—	—	100.0	85.0	10.6	4.4	0.000	0.002	0.071	
UFA (n = 29)	C10:1; C12:1n-1; C13:1n-1; C14:1n-5; C15:1n-5; C16:1n-7; C17:1n-7; Σ C18:1; C18:2 <i>c9 t11</i> ; C18:2 <i>t10 c11</i> ; C18:2n-6; C18:3n-6; C18:3n-3; C18:4n-3; C20:1n-12+15; C20:1n-9; C20:2n-6; C20:3n-6; C20:4n-6; C21:1n-9; C20:3n-3; C20:5n-3; C22:1n-9; C22:2n-6; C22:4n-6; C22:3n-3; C22:5n-3; C22:6n-3; C24:1n-9.	9	10	9	10	—	—	—	—	100.0	62.0	31.6	6.4	0.000	0.002	0.097	
LCFA ≥ 18 (n = 30)	C18:0 <i>iso</i> ; C18:0 <i>anteiso</i> ; C18:0; Σ C18:1; C18:2 <i>c9 t11</i> ; C18:2 <i>t10 c11</i> ; C18:2n-6; C18:3n-6; C18:3n-3; C18:4n-3; C20:0; C20:1n-12+15; C20:1n-9; C20:2n-6; C20:3n-6; C20:3n-3; C20:5n-3; C21:0; C20:4n-6; C22:0; C22:1n-9; C22:2n-6; C23:0; C22:4n-6; C22:3n-3; C22:5n-3; C24:0; C22:6n-3; C24:1n-9.	9	10	9	10	—	—	—	—	100.0	56.6	27.5	15.9	0.000	0.001	0.034	

¹FA = fatty acids; SFA = saturated fatty acids; UFA = unsaturated fatty acids; LCFA = long-chain fatty acids.

²*c* = *cis*; *t* = *trans*.

³Wilk's lambda is the ratio of the within-groups sum of squares and its value ranges from 0.0 to 1.0. Low values indicate strong group differences and values close to 1.0 no differences. Tests of function labeled 1-2 and 2-3 test the hypothesis that the means of the 3 functions are equal in the groups. Test 3 is a successive test useful to identify whether the additional function reflects population differences or only random variation.

sums of squares. For this reason, only variables with the highest coefficient values were effectively included in the models. Individual milk samples are plotted in terms of their values on these functions (discriminant scores) to give the best 2-dimensional graphical appraisal of the distinctiveness of the groups. The group means (centroids) are also shown.

Considering the multivariate test values (Wilk's lambda for test function) and the percentage of correct classifications, all considered discriminant criteria resulted in separation of the 4 groups (Table 6). However, in observing the scatter plot (Figure 1) of the scores of the first 2 functions, it appears that the 4 clusters are best discriminated using UFA and LCFA ≥ 18 , this latter group including SFA, MUFA, n-6 PUFA, n-3 PUFA, and CLA compounds. The UFA and LCFA ≥ 18 originate mainly from adsorption of dietary lipids from the digestive tract and from mobilization of body reserves (Bernard et al., 2006), and they better reflect in milk the different composition of rations caused by the variability of F:C or to the different composition of pasture according to territorial and annual climatic variations.

CONCLUSIONS

The FA composition of cow milk sampled in Italian and Slovenian farms during years 2005 and 2006 differed significantly depending more on country of origin than on year of production. The differences were likely attributable to the composition of animal diets. Fatty acids, especially UFA and LCFA ≥ 18 , appeared to be efficient markers for the discrimination of milk samples based on country of origin, even though discriminant functions probably reflect differences in forage and silage composition, animal genetics, and indigenous microflora. Moreover, fatty acid profile can be exploited as a method to discriminate between 2 (or more) areas of origin of the milk. The statistical models were developed using a relatively small number of milk samples; therefore, further research for validation of our findings, using a larger number of dairy farms located in different geographical areas, is required. To provide strong support for practical application of the proposed method, cross validation with blind samples collected from the 2 areas and analyzed for FA profile is needed to calculate the success of allocations. Finally, to confirm the validity of the markers, other studies should focus on factors (animal breed and age, lactation period, production level, farming system) other than feeds that could influence variation in the fatty acid composition of milk.

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